

Attorney Docket No.: 016252-002110US (AF-0019)  
Inventors: Graham et al.  
Serial No.: 10/032,658  
Filing Date: November 8, 2001  
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This listing of the claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

Claims 1-35 (canceled)

Claim 36 (currently amended): An isolated or recombinantly expressed antifreeze protein, said protein comprising the following:

(i) a calculated molecular weight of between 7 and 13 kDa;  
(ii) a thermal hysteresis activity of greater than 1.5°C at a concentration of about 1 mg/mL;

(iii) the N-terminal amino acid motif set forth in SEQ ID NO:3;

~~(iv) specific binding to an antibody raised against an antifreeze protein of YL-1 (SEQ ID NO:11); and~~

~~—(v) (vi) at least 70% amino acid sequence identity to an antifreeze protein of YL-1 (SEQ ID NO:11); and~~

(v) at least 4 repeats of the 12 contiguous amino acid motif set forth in SEQ ID NO:1.

Claim 37 (canceled)

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Claim 38 (currently amended): The isolated or recombinant antifreeze protein of ~~claim 37~~ claim 36, wherein the number of repeats of the motif is from 5 to 12.

Claim 39 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the calculated molecular weight of the antifreeze protein is between 8 and 12 kDa.

Claim 40 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein includes the subsequence of amino acids set forth in SEQ ID NO:4.

Claim 41 (previously amended): The isolated or recombinant antifreeze protein of claim 36, wherein the thermal hysteresis activity is greater than 2°C at a concentration of about 1 mg/mL.

Claim 42 (previously amended): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is YL-1 (SEQ ID NO:11).

Claim 43 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is expressed

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by a baculovirus vector.

Claim 44 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is synthesized by a bacterial cell, a fungus cell, a plant cell, or an animal cell.

Claim 45 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is synthesized by a yeast cell.

Claim 46 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is synthesized by an animal cell.

Claim 47 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the nucleic acid encoding the antifreeze protein is synthesized by an insect cell.

Claim 48 (original): The isolated or recombinant antifreeze protein of claim 36, wherein the antifreeze protein is derived from *Tenebrio* sp.

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Claim 49 (original): The isolated or recombinant antifreeze protein of claim 44, wherein the antifreeze protein is expressed externally from the cell.

Claims 50-77 (canceled)

Claim 78: (currently amended) A liquid comprising a recombinant antifreeze protein, said antifreeze protein comprising the following:

- (i) a calculated molecular weight of between 7 and 13 kDa;
- (ii) a thermal hysteresis activity of greater than 1.5°C at a concentration of about 1 mg/mL;
- (iii) the N-terminal amino acid motif set forth in SEQ ID NO:3;
- ~~(iv) specific binding to an antibody raised against an antifreeze protein of YL-1 (SEQ ID NO:11); and~~
- ~~— (v) (iv) at least 70% amino acid sequence identity to an antifreeze protein of YL-1 (SEQ ID NO:11); and~~
- (v) at least 4 repeats of the 12 contiguous amino acid motif set forth in SEQ ID NO:1.

Claim 79 (original): The liquid of claim 78, wherein the

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antifreeze protein comprises at least one repeat of the 12  
contiguous amino acid motif set forth in SEQ ID NO:1.

Claim 80 (original): The liquid of claim 78, wherein the  
concentration of antifreeze protein is between about one part per  
billion (1 µg/L) to about one part per thousand (1 g/L).

Claim 81 (previously amended): The liquid of claim 78,  
wherein the thermal hysteresis activity is greater than 2°C at a  
concentration of about 1 mg/mL.